

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant: Indu Bhusan Chatterjee

Serial No.: Not Yet Assigned

For: A Process for the Isolation of a Major Harmful Oxidant from Cigarette Smoke

Filed: Not Yet Assigned

Examiner: Not Yet Assigned

Art Unit: Not Yet Assigned

Attorney Docket No. 3030.003USU

Box Patent Application  
Assistant Commissioner of Patents  
Washington, D.C. 20231

February 13, 2002

Sir:

**PRELIMINARY AMENDMENT**

Applicants respectfully submit the following amendments and remarks.

**IN THE CLAIMS:**

Please amend the claims as follows:

3. (Amended) A process as claimed in claim 1, wherein said isolated pure cigarette smoke (cs) oxidant has the following properties:
  - (a) when crystallized from acetone solution appears as small needle shaped faint yellow coloured crystals having pungent smell, similar to that of rancid butterfat,
  - (b) UV absorption maxima in methanol solution are at 293.4 nm and 223.0 nm and in aqueous solution are in 288nm and 221nm, respectively,
  - (c) on excitation at 293 nm in methanol solution the observed emission maxima are at 329.6 nm and 651.4 nm and on excitation at 224 nm, the observed emission maxima are at 329.6 nm and 652.6 nm, respectively,

- (d) when excitation scanning is monitored keeping the emission at 330 nm, the observed excitation maxima are at 228.2 nm and 293.8 nm and when the emission is kept at 651 nm and excitation scanning is monitored, the observed excitation maxima are at 229.2 nm and 294.8 nm, respectively,
- (e) highly soluble in methanol, ethanol, acetone, n-butanol, fairly soluble in water, sparingly soluble in methylene chloride, di-ethyl ether, chloroform and insoluble in benzene and petroleum ether,
- (f) the compound loses its oxidizing potency in acidic pH ranging between 4 to 5 and on keeping the solution at alkaline pH ranging between 9 to 10, the compound gradually turns brown, at pH 10 and above there is instantaneous darkening with loss of both activity and aromaticity as evidenced by UV spectroscopy,
- (g) the half-life of the oxidant, when stored in the solid state at a temperature ranging between 25 °C to 30 °C under darkness is about 48 hours as determined by its oxidative potency, but in solution of 50 mM potassium phosphate buffer, pH 7.4 at 25°C to 30°C the half life is about 1 hour 30 min,
- (h) reduces ferricytochrome c and ferric chloride,
- (i) oxidizes ascorbic acid, proteins and DNA, and
- (j) the melting point is 162°C.

6. (Amended) A process as claimed in claim 1, wherein p-benzosemiquinone present in cs solution is quantitatively assayed by HPLC with a UV detector using a 25 cm reverse phase ODS column and using a mixture of water and methanol (95: 5 v/v) as a mobile phase, at a wave length of 288nm, flow rate of 0.8 ml/min, at a temperature of about 25°C and at a pressure of about 147 Kgf/cm<sup>2</sup> and having a retention time of 13.46 min.

Please delete claims 36 and 37, without prejudice or disclaimer.

Please add the following new claims:

39. A process as claimed in claim 2, wherein said isolated pure cigarette smoke (cs) oxidant has the following properties:

- (a) when crystallized from acetone solution appears as small needle shaped faint yellow coloured crystals having pungent smell, similar to that of rancid butterfat,
- (b) UV absorption maxima in methanol solution are at 293.4 nm and 223.0 nm and in aqueous solution are in 288nm and 221nm, respectively,
- (c) on excitation at 293 nm in methanol solution the observed emission maxima are at 329.6 nm and 651.4 nm and on excitation at 224 nm, the observed emission maxima are at 329.6 nm and 652.6 nm, respectively,
- (d) when excitation scanning is monitored keeping the emission at 330 nm, the observed excitation maxima are at 228.2 nm and 293.8 nm and when the emission is kept at 651 nm and excitation scanning is monitored, the observed excitation maxima are at 229.2 nm and 294.8 nm, respectively,
- (e) highly soluble in methanol, ethanol, acetone, n-butanol, fairly soluble in water, sparingly soluble in methylene chloride, di-ethyl ether, chloroform and insoluble in benzene and petroleum ether,
- (f) the compound loses its oxidizing potency in acidic pH ranging between 4 to 5 and on keeping the solution at alkaline pH ranging between 9 to 10, the compound gradually turns brown, at pH 10 and above there is instantaneous darkening with loss of both activity and aromaticity as evidenced by UV spectroscopy,
- (g) the half-life of the oxidant, when stored in the solid state at a temperature ranging between 25 °C to 30 °C under darkness is about 48 hours as determined by its oxidative potency, but in solution of 50 mM potassium phosphate buffer, pH 7.4 at 25°C to 30°C the half life is about 1 hour 30 min,
- (h) reduces ferricytochrome c and ferric chloride,
- (i) oxidizes ascorbic acid, proteins and DNA, and
- (j) the melting point is 162°C.

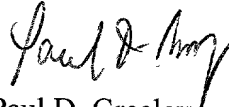
40. A process as claimed in claim 2, wherein p-benzosemiquinone present in cs solution is quantitatively assayed by HPLC with a UV detector using a 25 cm reverse phase ODS column and using a mixture of water and methanol (95: 5 v/v) as a mobile phase, at a wave length of 288nm, flow rate of 0.8 ml/min, at a temperature of about 25°C and at a pressure of about 147 Kgf/cm<sup>2</sup> and having a retention time of 13.46 min.
41. A process as claimed in claim 4, wherein p-benzosemiquinone present in cs solution is quantitatively assayed by HPLC with a UV detector using a 25 cm reverse phase ODS column and using a mixture of water and methanol (95: 5 v/v) as a mobile phase, at a wave length of 288nm, flow rate of 0.8 ml/min, at a temperature of about 25°C and at a pressure of about 147 Kgf/cm<sup>2</sup> and having a retention time of 13.46 min.

#### **REMARKS**

Claims 3 and 6 have been clarified by amendment above for purposes of removing improper multiple dependencies therefrom. Claims 36 and 37 have been deleted, without prejudice or disclaimer, since these type of use claims are not proper under U.S. patent practice. New claims 39-41 correspond to claims 3 and 6, but depend upon other independent claims. It is respectfully submitted that the amendments to the claims are neither narrowing nor made for substantial reasons related to patentability as defined by the Court of Appeals for the Federal Circuit (CAFC) in Festo Corporation v. Shoketsu Kinzoku Kogyo Kabushiki Co., Ltd., 95-1066 (Fed. Cir. 2000). Therefore, the amendments to the claims do not create prosecution history estoppel and, as such, the doctrine of equivalents is available for all of the elements of the claim.

In view of the foregoing amendments and remarks applicant respectfully requests reconsideration and allowance of all the claims presently in the application.

Respectfully submitted,

A handwritten signature in black ink, appearing to read "Paul D. Greeley", with a long, sweeping horizontal stroke extending to the right.

Paul D. Greeley  
Ohlandt, Greeley, Ruggiero & Perle  
Attorneys for Applicant(s)  
Reg. No. 31,019  
(203)327-4500

## VERSION WITH MARKINGS TO SHOW CHANGES MADE

### IN THE CLAIMS:

Please amend the claims as follows:

4. (Amended) A process as claimed in ~~claims 1&2~~ claim 1, wherein the said isolated pure cigarette smoke (cs) oxidant has the following properties:
- (k) when crystallized from acetone solution appears as small needle shaped faint yellow coloured crystals having pungent smell, similar to that of rancid butterfat,
  - (l) UV absorption maxima in methanol solution are at 293.4 nm and 223.0 nm and in aqueous solution are in 288nm and 221nm, respectively,
  - (m) on excitation at 293 nm in methanol solution the observed emission maxima are at 329.6 nm and 651.4 nm and on excitation at 224 nm, the observed emission maxima are at 329.6 nm and 652.6 nm, respectively,
  - (n) when excitation scanning is monitored keeping the emission at 330 nm, the observed excitation maxima are at 228.2 nm and 293.8 nm and when the emission is kept at 651 nm and excitation scanning is monitored, the observed excitation maxima are at 229.2 nm and 294.8 nm, respectively,
  - (o) highly soluble in methanol, ethanol, acetone, n-butanol, fairly soluble in water, sparingly soluble in methylene chloride, di-ethyl ether, chloroform and insoluble in benzene and petroleum ether,
  - (p) the compound loses its oxidizing potency in acidic pH ranging between 4 to 5 and on keeping the solution at alkaline pH ranging between 9 to 10, the compound gradually turns brown, at pH 10 and above there is instantaneous darkening with loss of both activity and aromaticity as evidenced by UV spectroscopy,
  - (q) the half-life of the oxidant, when stored in the solid state at a temperature ranging between 25 °C to 30 °C under darkness is about 48 hours as determined by its oxidative potency, but in solution of 50 mM potassium phosphate buffer, pH 7.4 at 25°C to 30°C the half life is about 1 hour 30 min,

- (r) reduces ferricytochrome c and ferric chloride,
- (s) oxidizes ascorbic acid , proteins and DNA, and
- (t) the melting point is 162°C;

7. (Amended) A process as claimed in ~~claims 1 to 4~~ claim 1, wherein p-benzosemiquinone present in cs solution is quantitatively assayed by HPLC with a UV detector using a 25 cm reverse phase ODS column and using a mixture of water and methanol (95: 5 v/v) as a mobile phase, at a wave length of 288nm, flow rate of 0.8 ml/min, at a temperature of about 25°C and at a pressure of about 147 Kg/cm<sup>2</sup> and having a retention time of 13.46 min.

Please delete claims 36 and 37, without prejudice or disclaimer.

Please add the following new claims:

42. A process as claimed in claim 2, wherein said isolated pure cigarette smoke (cs) oxidant has the following properties:
- (k) when crystallized from acetone solution appears as small needle shaped faint yellow coloured crystals having pungent smell, similar to that of rancid butterfat,
  - (l) UV absorption maxima in methanol solution are at 293.4 nm and 223.0 nm and in aqueous solution are in 288nm and 221nm, respectively,
  - (m) on excitation at 293 nm in methanol solution the observed emission maxima are at 329.6 nm and 651.4 nm and on excitation at 224 nm, the observed emission maxima are at 329.6 nm and 652.6 nm, respectively,
  - (n) when excitation scanning is monitored keeping the emission at 330 nm, the observed excitation maxima are at 228.2 nm and 293.8 nm and when the emission is kept at 651 nm and excitation scanning is monitored, the observed excitation maxima are at 229.2 nm and 294.8 nm, respectively,
  - (o) highly soluble in methanol, ethanol, acetone, n-butanol, fairly soluble in water, sparingly soluble in methylene chloride, di-ethyl ether, chloroform and

insoluble in benzene and petroleum ether,

- (p) the compound loses its oxidizing potency in acidic pH ranging between 4 to 5 and on keeping the solution at alkaline pH ranging between 9 to 10, the compound gradually turns brown, at pH 10 and above there is instantaneous darkening with loss of both activity and aromaticity as evidenced by UV spectroscopy,
- (q) the half-life of the oxidant, when stored in the solid state at a temperature ranging between 25 °C to 30 °C under darkness is about 48 hours as determined by its oxidative potency, but in solution of 50 mM potassium phosphate buffer, pH 7.4 at 25°C to 30°C the half life is about 1 hour 30 min,
- (r) reduces ferricytochrome c and ferric chloride,
- (s) oxidizes ascorbic acid, proteins and DNA, and
- (t) the melting point is 162°C.

43. A process as claimed in claim 2, wherein p-benzosemiquinone present in cs solution is quantitatively assayed by HPLC with a UV detector using a 25 cm reverse phase ODS column and using a mixture of water and methanol (95: 5 v/v) as a mobile phase, at a wave length of 288nm, flow rate of 0.8 ml/min, at a temperature of about 25°C and at a pressure of about 147 Kgf/cm<sup>2</sup> and having a retention time of 13.46 min.

44. A process as claimed in claim 4, wherein p-benzosemiquinone present in cs solution is quantitatively assayed by HPLC with a UV detector using a 25 cm reverse phase ODS column and using a mixture of water and methanol (95: 5 v/v) as a mobile phase, at a wave length of 288nm, flow rate of 0.8 ml/min, at a temperature of about 25°C and at a pressure of about 147 Kgf/cm<sup>2</sup> and having a retention time of 13.46 min.